Fourier Transform Infrared Investigation of Non-Heme Fe(III) and Fe(II) Decomposition of Artemisinin and of a Simplified Trioxane Alcohol

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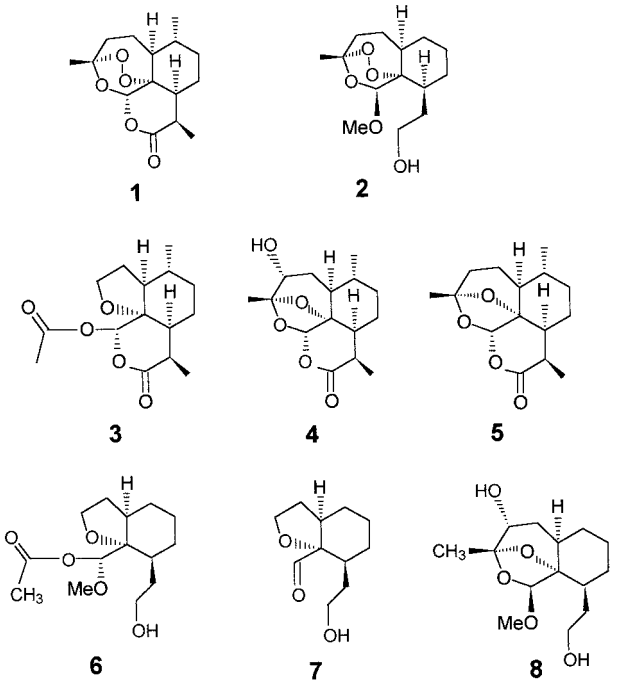
Fourier transform infrared spectra are reported for the Fe(III)- and Fe(II)-mediated activation of the antimalarial agents artemisinin **1** and its simplified synthetic analogue, trioxane alcohol **2**. By monitoring the frequencies of the newly established marker lines in the FTIR spectra, the products of the Fe(II) and Fe(III) reactions have been characterized. In both reactions, artemisinin is activated giving a product mixture of a ring-contracted tetrahydrofuran acetatal **3**, C_4-hydroxy deoxyartemisinin **4**, and deoxyartemisinin **5**. These data illustrate that the oxidation state of the iron places no restrictions on the endoperoxide reduction mechanism. The FTIR difference (light − dark) spectra indicate that the endoperoxide moiety of artemisinin is photolabile and that the resulted products have the same vibrational characteristics as those observed in the reactions with Fe(II) and Fe(III). The use of ^{18}O-^{18}O enriched endoperoxide in **2** has allowed us to identify two oxygen sensitive modes in the reactions with Fe(II). The reduction of the peroxide bond by Fe(II) in trioxane alcohol **2** follows both the C−C cleavage and 1,5-H shift pathways and produces a ring-contracted tetrahydrofuran acetal **6** which is converted to tetrahydrofuran aldehyde **7** and C_4-hydroxy deoxytrioxane alcohol **8**, respectively. The cleavage of the O−O bond in **1** and **2** by iron and the ability to correlate vibrational properties of the reaction products with structural properties of the isolated products suggest that infrared spectroscopy is an appropriate tool to study the mode of action of antimalarial endoperoxides.

Artemisinin **1** (Chart 1) is a novel natural sesquiterpene lactone (1,2,4-trioxane), isolated from *Artemisia annua,* with an endoperoxide function essential in the chemotherapy of chloroquine-resistant strains of *Plasmodium falciparum* malaria. The mode of action of artemisinin and its simplified trioxane analogues is at the intraerythrocytic stage.[1−4] The heme groups released from the digestion of hemoglobin of infected blood cells are aggregated into an insoluble substance called hemozoin.[5] Recently, the crystal structure of β-hematin, which was shown to be essentially hemozoin, was reported.[4] The spread of *P. falciparum* has initiated a tremendous interest in the mechanism of action, drug development, and biochemistry behind the antimalarial activity of artemisinin, and other structurally related synthetic 1,2,4-trioxanes. Á number of tetracyclic artemisinin derivatives and tricyclic 1,2,4-trioxanes have been developed as potent and rapidly acting antimalarials.[7−17] Despite intensive research efforts, neither the mechanism of activating these drugs into cytotoxic species nor the centers and their redox state associated with the linkage have yet been identified. It has been accepted, however, that the different iron pools, such as free iron and heme, within the malarial parasite, are responsible for activating the 1,2,4-trioxane antimalarials to form cytotoxic radical intermediates.[18,19]

The mode of action of artemisinin has received a great deal of attention in recent years as it allows for the clarification of the molecular mechanisms in this class of drugs and the rationalization of antimalarial activity in others. Despite complexities apparently caused by differences in the choice of experimental approach by several research groups, some common properties were found. On the basis of these experiments, structures for many intermediates have been proposed, and the rates for the associated O−O cleavage steps have been determined. Some of the structures were inferred based

Chart 1



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on observed final products, and others were proposed based on spectroscopic properties. On the basis of Fe(II)-induced decomposition of artemisinin and other trioxanes, it has been suggested that the cleavage of the O−O bond of the endoperoxide moiety is the initial key step in the activation of the drug.[7−17] In such a way, the cleavage of the endoperoxide generates oxygen-centered radicals that immediately rearrange to carbon centered radicals. The transformation from the oxygen- to carbon-centered radicals can occur either from a homolytic cleavage of the C_3-C_4 bond or an intramolecular 1,5-H atom shift. In recent studies of Posner and co-workers, the major products of the Fe(II)-induced activation of artemisinin were found to be compounds **3**, **4**, and **5** in 29%, 15%, and 54% yields, respectively.[4] Compounds **3** and **4** are pyrolysis products of artemisinin.[20] Compound **4** is also a known microbial metabolite of artemisinin,[21,22] and compound **5** is both a microbial and mammalian metabolite.[22] More recently, Wu and co-workers in similar experiments isolated and characterized compounds **3** and **4** in 25% and 67% yields, respectively.[23] In addition, they were able to isolate an unstable epoxide that was postulated in earlier studies by Posner et al. as the species responsible for the antimalarial activity of the drug,[24] and also they trapped and characterized a secondary C-4 radical. The latter observation provides the first direct evidence for the involvement of radicals in the reductive cleavage of the trioxane-type antimalarial compounds. Very interestingly, deoxyartemisinin **5** was not obtained at all, and its absence was attributed to the major difference between the reaction conducted in aqueous medium and that in THF. They also reported that no reaction occurred when $FeSO_4$ was replaced by $FeCl_3$.[23] Haynes and Vonwiller,[25] however, isolated and characterized compounds **3** and **4** in the reactions with both $FeSO_4$ and $FeCl_3$. These studies led to a consensus on the structures of compounds **3**, **4**, and **5** and puzzling disagreements on the main product ratios and the mechanism for the endoperoxide reduction by Fe(II). The analogous reactions with Fe(III), however, have received little attention, and a number of questions concerning the structures of the activation products and the mechanism of the critical O−O bond cleavage process are ill answered.

Several important properties of artemisinin have been determined from studies of simpler trioxane analogues. It has been established by Posner and co-workers that deoxygenation of 1,2,4-trioxanes into corresponding 1,3-dioxolanes occurs via an unzipping-zipping process.[4] The Fe(II)-induced cleavage of the peroxide bond in trioxane tosylate leads through the C−C bond cleavage to a ring-contracted tetrahydrofuran acetal **6** and then produces a stable electophilic tetrahydrofuran aldehyde **7**. On the other hand, the 1,5-H atom abstraction process produces a stable C_4-hydroxy deoxytrioxane alcohol **8** as a mixture of two diastereomers. These findings led them to conclude that the reduction of the endoperoxide by Fe(II) follows a different mechanistic course that leads to different products than the endoperoxide cleavage by non Fe(II) reducing agents.[8] Recently, the use of the $^{18}O_2$ enriched trioxane alcohol allowed us to detect the heme Fe(IV)═O intermediate in the artemisinin/hemin dimer reaction.[26]

The Fourier transform infrared (FTIR) technique has been applied to the study of some 1,2,4-trioxanes and has been found to be powerful in probing the bonds of the endoperoxide moiety and the bonds of the rings owing to the presence of the O−O, the C−O, the O−O−C, as well as the C═O modes in the spectrum. The endoperoxide moiety is especially useful in this regard because the homolytic cleavage of the O−O bond can be characterized and hence can be used to assess the vibrational properties of the O- and C-centered radicals and subsequently that of the C_3-C_4 bond cleavage.

In the work presented here, we have applied the FTIR approach to further characterize the reactions of artemisinin and its synthetic analogue trioxane alcohol with Fe(II) and Fe(III). Compounds **3**, **4**, and **5** are detected in the Fe(II)/artemisinin reaction. They are readily characterized by the $-COOCH_3$ mode at 1717 cm^{-1} (compound **3**), the hydroxyl mode at 3440 cm^{-1} (compound **4**), and the six-membered lactone at 1755 cm^{-1} (compound **5**). Although the Fe(III)/artemisinin reaction is slower, we show cleavage of the O−O bond and the formation of three products which have vibrational characteristics similar to those found in the products of the Fe(II)/artemisinin reaction. The product ratios in the Fe(II) and Fe(III) reactions depends on the oxidation state of the iron and thus on whether the activation follows the 1,5 H-shift or the C_3-C_4 cleavage pathway. In the case of the Fe(II)-induced cleavage of trioxane alcohol **2**, three major products are detected, and their characteristic C═O vibrations are located at 1717 and 1740 cm^{-1} and that of the hydroxyl (OH) at 3433 cm^{-1}. From consideration of determined structures, we infer these frequencies as being associated with compounds **6**, **7**, and **8**. We have also applied laser photolysis FTIR techniques to monitor the FTIR spectra of artemisinin photoproducts and found that the O−O bond is photolabile. The artemisinin photoproducts have vibrational characteristics that are similar to those observed in their corresponding Fe(II)- and Fe(III)-induced reductive cleavage products. From the different product ratios observed in the Fe-induced cleavage of the endoperoxide and that in the photolytic cleavage, we infer that there is a mechanistic difference between the two processes. These results also provide important links between metabolites and chemical reaction products that have been observed in the antimalarial mode of action of trioxanes.

Materials and Methods

Artemisinin, ferrous bromide, and ferric chloride (Aldrich Chemical Co.) were used as purchased. Trioxane alcohol was synthesized according to Posner et al.[7] The oxygen-18 labeled trioxane alcohol was synthesized by introducing $^{18}O_2$ during the photooxygenation step. The $^{18}O_2$ was a product of Isotec. All reactions were carried out in acetonitrile (CH_3CN) containing 1 equiv of either $FeBr_2$ or $FeCl_3$. The FTIR spectra were recorded from thin film samples at 2 cm^{-1} resolution with a Bruker Equinox 55 FTIR spectrometer equipped with a liquid nitrogen cooled mercury cadmium telluride detector. The photolysis experiments were performed with a Nd:YAG laser, 532 nm, ten 10 mJ flashes, 7 ns duration.

Results and Discussion

The FTIR spectra presented here and the frequencies of the modes are fully consistent with earlier studies of artemisinin and with the vibrational data reported for

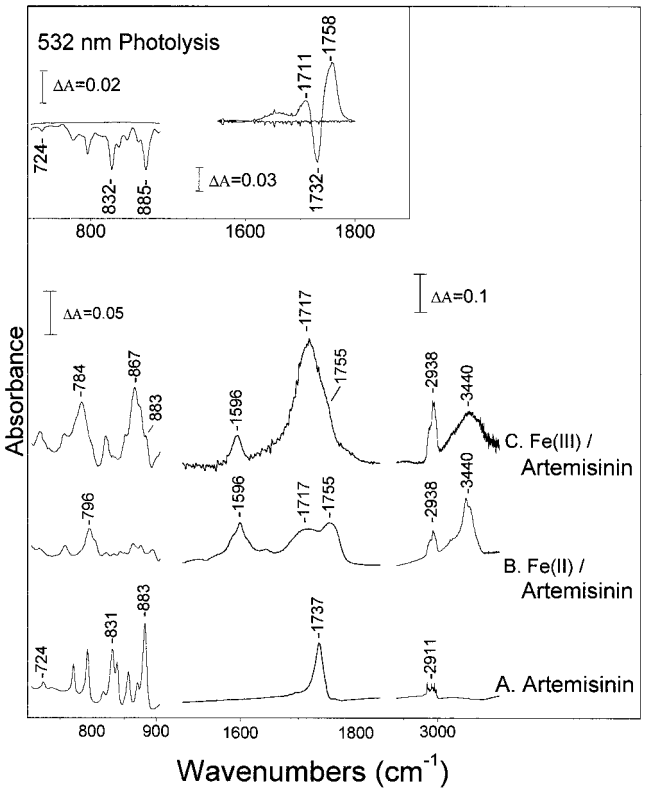

Figure 1. FTIR spectra of artemisinin (trace A), Fe(II)/artemisinin reaction (trace B), and Fe(III)/artemisinin reaction (trace C). The inset shows the "light − dark" FTIR spectrum of artemisinin.

some 1,2,4-trioxanes by Jefford and co-workers.[17] The FTIR modes in the 800−1200 cm^{-1} range have been shown to be sensitive indicators to the O−O and C−O modes of the O−O−C unit, and at 1737 cm^{-1} to the δ-lactone carbonyl ν(C═O) mode. It has been shown from previous experiments that addition of Fe(II) to artemisinin leads to the formation of compound **3** and either compound **4** or **5** which have been isolated and characterized by their C═O and OH vibrations. The FTIR spectra[23] of the isolated product **3** exhibit the δ-lactone carbonyl, ν(C═O) mode at 1760 cm^{-1} while the analogous ν(C═O) of the isolated products **4**[23] and **5**[14] are located at 1730 and 1742 cm^{-1}, respectively. In compound **4**, the ν(OH) is located at 3050 cm^{-1} and the C═O mode of the $-COOCH_3$ moiety of compound **3** at 1746 cm^{-1}.[23] The isolated products from the Fe(II)/trioxane tosylate reaction exhibit the C═O mode of the $-COOCH_3$ moiety of compound **6** at 1726 cm^{-1}, the C═O mode of compound **7** at 1735 cm^{-1}, and the OH mode of compound **8** at 3400 cm^{-1}.[8] The FTIR spectra we report here allow for determination of the final products and characterization of their vibrational properties. We assign the bands in the 1700−1760 and 3400 cm^{-1} regions as originating from C═O modes (1700 cm^{-1} for ketones and 1755 cm^{-1} for lactones or saturated esters) and OH modes, respectively, and thus utilize them as marker lines for product formation since spectral overlap with other sharp contributions in this spectral range is not large.

Figure 1A shows the FTIR spectra of artemisinin, and those of the Fe(II)- and Fe(III)-induced reaction products are shown in Figure 1B,C, respectively. The principal bands seen in Figure 1A are assigned as analogous to those vibrations found in other 1,2,4-trioxanes.[17] The O−O and C−O stretching modes of the O−O−C moiety are located at 724, 831, and 883 cm^{-1}, respectively, and the C═O of δ-lactone is located at 1737 cm^{-1}. Figure 1B,C shows that extensive changes have occurred in the vibrational properties of artemisinin in the reactions with Fe(II) and Fe(III). In the low-frequency region of the spectrum, the lines at 831 and 883 cm^{-1} have disappeared, an expected characteristic of the endoperoxide cleavage. We find five new bands located at 1596, 1717, 1755, 2938, and 3440 cm^{-1} in the high-frequency region of the FTIR spectra. The line at 1717 is assigned as the C═O stretching mode of the $-COOCH_3$ moiety of compound **3**. We assign the line at 1755 as originating from the C═O of compound **5**. Finally, the line at 3440 cm^{-1} is attributed to OH mode of compound **4**. These results illustrate the independence of the reaction products on the oxidation state of the iron and hence indicate that all the features in the spectra result from the cleavage of the O−O bond. However, the relative proportions of the 1755 and 1717 cm^{-1} species in the Fe(III)/artemisinin reaction are different from that detected in the Fe(II)/artemisinin reaction. Thus, two separate pathways, as discussed below, are involved in the activation products of artemisinin by iron.

To further investigate the artemisinin activation process we have monitored the FTIR spectra of artemisinin after 532 nm laser pulsed excitation. The FTIR difference spectra (light − dark) presented as an inset of Figure 1 show two peaks at 1711 and 1758 cm^{-1} as well as three troughs at 724, 832, 885, and 1732 cm^{-1}. This observation suggests that the O−O bond is photolabile and that new species are formed after the endoperoxide cleavage. The observed spectrum is characteristic of compounds **3** and **5**. This is indicated by the lines at 1711 and 1758 cm^{-1}. Thus, in the absence of either Fe(II) or Fe(III), the photolytic cleavage of the O−O bond is also capable to convert artemisinin to compounds **3** and **5**. Interestingly, the intensity ratio of the 1758 /1711 cm^{-1} modes suggests that product ratio is similar to that measured in the Fe(II) reaction.

To gain further insight into the endoperoxide chemistry and thus in the mode of action of artemisinin, we have studied the artemisinin analogue trioxane alcohol ($IC_{50} \sim 34$ ng/mL,[28] concentration of the drug required for killing 50% of *P. falciparum* in culture). The use of this compound and other related trioxanes has allowed Posner and co-workers to propose the 1,5-H atom shift and C−C cleavage processes that have been widely accepted. In contrast to artemisinin, which has a characteristic vibration at 1737 cm^{-1} due to the carbonyl group of the lactone moiety, the trioxane alcohol **2** lacks vibrations in this region because of the absence of any C═O groups. The FTIR spectra of the Fe(II)-reductive cleavage of **2** were measured for both $^{16}O_2$ and $^{18}O_2$ enriched endoperoxides. The FTIR spectra (Figure 2A) of the final products of the ^{16}O-^{16}O enriched endoperoxide reaction with Fe(II) exhibit peaks at 1717 and 1740 cm^{-1}. Figure 2B shows that the 1717 cm^{-1} mode in the ^{16}O-^{16}O has lost intensity and is downshifted to 1712 cm^{-1} in the ^{18}O-^{18}O derivative while the 1740 cm^{-1} mode remains unshifted. These results indicate that the 1717 cm^{-1} mode originates from species that contain groups that have resulted from the O−O cleavage. It

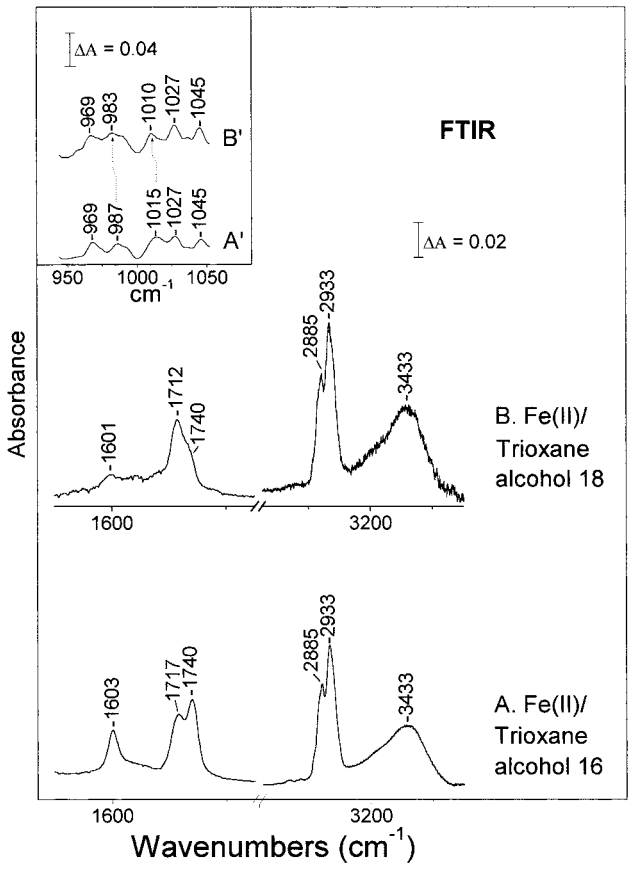

Figure 2. FTIR spectra of the Fe(II) reaction with trioxane alcohol in ^{16}O-^{16}O enriched endoperoxide (traces A and A′) and ^{18}O-^{18}O enriched endoperoxide (trace B and B′).

has been reported that the C═O modes of the tosylate derivatives of compound **6** and **7** are at 1726 and 1735 cm^{-1}, respectively.[8] The carbonyl mode of compound **7** is not a consequence of the endoperoxide cleavage and thus is not expected to show isotope frequency shift. On the other hand, the 1726 cm^{-1} carbonyl mode originates from the endoperoxide cleavage and therefore is expected to show isotope frequency shift. We adopt the assignment and mechanism offered by Posner and co-workers[4] that the 1717 and 1740 cm^{-1} modes we detect are associated with compounds **6** and **7**, respectively. We interpret the small isotope frequency shift at 1717 cm^{-1} as a result of vibrational coupling between the C═O mode and the modes of compound **6**. The broad shape and intensity of the OH mode at 3433 cm^{-1} of compound **2** in the spectra of the final products makes it difficult to draw simple conclusions concerning the formation of compound **8**. Thus the OH mode is unable to serve as a probe of compound **8** since trioxane alcohol **2** possesses already a hydroxyl group. However, the observed low-frequency data shown in the inset exhibit an oxygen isotope shift pattern in the 900−1000 cm^{-1} region. The oxygen-sensitive lines at 987 and 1015 cm^{-1} are not unique features only seen in the products of **2** but are common C−O−C modes found in other ring complexes containing the C−O−C unit. It has been demonstrated that as the ring becomes smaller, the frequency of the band involving the asymmetric stretch of the C−O−C bonds decreases. We interpret the observed shift pattern at 987 cm^{-1} as originating from the asymmetric C−O−C stretching vibrations of compounds **6** and **7** and that at 1015 cm^{-1} to compound **8**.

On the basis of the above results, it now appears very likely that the electron transfer from Fe(II) to the endoperoxide in **1** has cleaved the O−O bond and initiated the formation of radical intermediates which are rapidly converted to compounds **3**, **4**, and **5** as the major products.[8] Similar conclusions were previously drawn by Posner and co-workers.[4] However, Wu et al. reported the formation of compounds **3** and **4**, only.[23] It is clear from these experiments that the formation of compound **5** is dependent on the experimental conditions. It is detectable in the protocol used by Posner and co-workers or by the present authors but not in that used by Wu and co-workers.[23] Up to now, the endoperoxide cleavage of 1,2,4-trioxanes is agreed by most researchers in the field to be induced by Fe(II), without addressing the main products in the analogous reaction with Fe(III). The spectra shown in Figure 1C demonstrate that compounds **3**, **4**, and **5** are also formed in the Fe(III)/artemisinin reaction. This is in sharp contrast to the conclusions drawn by Wu et al.[23] They reported that no reaction occurs when the $FeSO_4$ is replaced with $FeCl_3$. Thus, their interpretation that the replacement of Fe^{2+} by other metal ions (M^{n+}) will retard both the β-scission and the radical substitution at the oxygen atom is inconsistent with our data. The main products reported by Haynes and Vonwiller,[25] however, who detected compounds **3** (85% yield) and **4** (8% yield) as the main products of the Fe(III)/artemisinin reaction, are in partial agreement with our data, and this further supports our assignment of the 1717 cm^{-1} being associated with compound **3**. The 1717 cm^{-1} band is much stronger than the 1755 cm^{-1} band, which suggests that compound **3** is the major reaction product. For a complete vibrational analysis of the Fe/artemisinin reaction products, a variety of isotopic substituted derivatives would be necessary, but the present data set is adequate for our purposes. It is clear from the experiments reported here that both Fe(II) and Fe(III) react with artemisinin to produce the same final products. There is general agreement that artemisinin activation products arise from the homolytic fission of the endoperoxide bond initiated by single electron donation from Fe(II). In the absence of electron transfer from Fe(III) to the endoperoxide, we postulate that precomplexation, rather than direct cleavage of the peroxide leads to formation of products (see below).

The formation of new products in the photolytic activation of artemisinin is not a surprising result since it is clear from our data as well as those of others that the activation of **1** progresses rapidly to the formation of compounds **3**, **4**, and **5**. The data reported by Lin and co-workers,[20] who fully characterized by IR, ^1H NMR, ^{13}C NMR, and X-ray crystallography the heat decomposition (190 °C) products of artemisinin, were interpreted as the formation of compounds **3** and **4**. They also proposed that the mechanism for the formation of these products involves the homolytic cleavage of the endoperoxide to generate a free radical intermediate that rearranges or decomposes to give the observed products. The comparison of our photolytic data to the reference data clearly illustrates that species are produced with spectra very similar to the spectrum shown in the inset

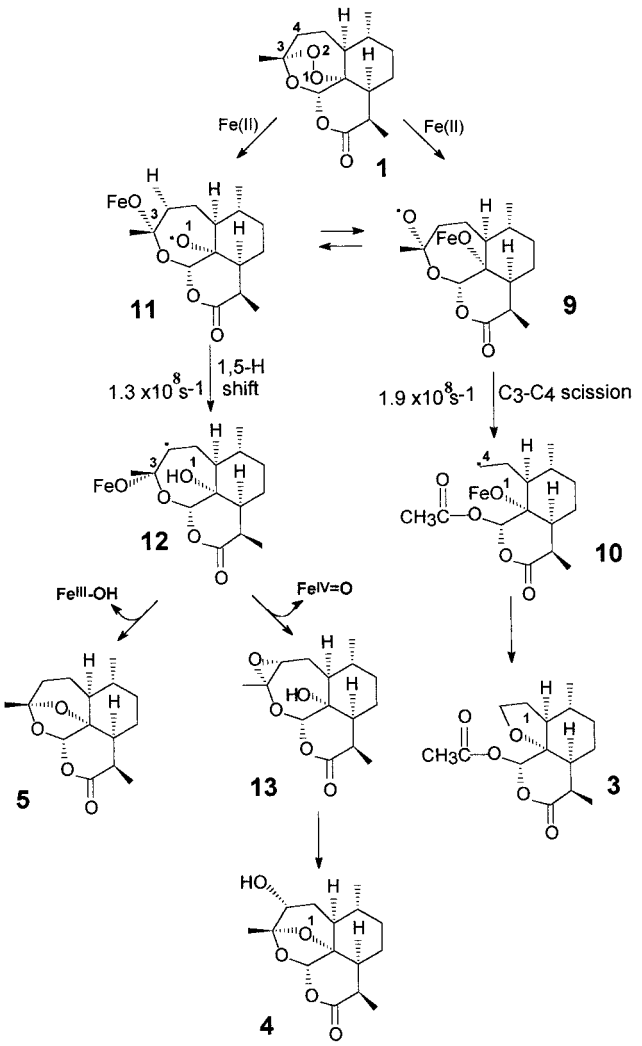

Figure 3. Proposed mechanism for the Fe(II)-induced activation of artemisinin.

of Figure 1, namely, compounds **3**, **4**, and **5**. We detect no change in the position of the lines in the 1700−1750 cm^{-1} region, as would be expected, from the transition of the Fe-induced decomposition to the photolytic decomposition of artemisinin. This observation suggests that the formation of the final products in artemisinin is independent of the way the O−O bond is cleaved.

In an effort to understand the progression of the intermediates in the O−O cleavage of artemisinin, we first present a reaction scheme for artemisinin in Figure 3. The structures of the intermediates and those of the final products were taken from the literature and from the present experiments. The data also show that the chemical conditions of the reactions determine which of the intermediates can accumulate. Although in the scheme depicted in Figure 3 we show O- and C-centered radicals, there is no evidence for the build-up of such intermediates in our measurements. The characterization of the O-centered radicals produced in the cleavage of the O−O bond as well as the detection of the produced C-centered radicals formed either through a 1,5-hydrogen atom shift or the homolytic C_3-C_4 bond cleavage remains problematic. It has been shown, however, in several 1,2,4-trioxanes such as artemisinin that, of the two pathways, only the one involving a C_4 radical intermediate through a 1,5-H shift is important for high antimalarial activity.[27,28] Recently, Gu et al.[29] in a DTF study showed that the rate constant for the classical 1,5-H shift is 1.3×10^8 s^{-1} and 2.9×10^{-35} s^{-1} at 298 and 30 K, respectively, and concluded that the corresponding O-centered radical is detectable experimentally at low temperature. Moreover, they calculated the C−C cleavage rate to be 1.9×10^8 s^{-1} and 1.2×10^{-33} s^{-1} at 298 and 30 K, respectively, and reported that the C-centered radical is 5.2 kcal/mol more stable than the O-centered radical. Since there is experimental evidence for the formation of a secondary C-4 radical and of an unstable epoxide by Wu et al.[23] and for the formation of a primary C-3 radical,[30] their structures were used in the scheme. The epoxide was characterized by its IR spectrum giving rise to ν(OH) and ν(C=O) at 3500 and 1728 cm^{-1}, respectively.

In the reaction scheme depicted in Figure 3, two separate pathways are indicated for the decay of the primary O-centered radicals. In the first pathway, if iron associates with O-1, then compound **9** is formed which is converted via the C_3-C_4 scission to the C-4 radical compound **10.** The latter compound is converted to compound **3**. On the other hand, if iron associates with O-2, then through the 1,5-H shift compound **11** is converted to the C-4 radical compound **12**. The later compound can be either converted to the unstable epoxide **13** with the concomitant formation of Fe(IV)=O or to the stable compound **5** and the formation of Fe(III)-OH. Compound **13** can be further converted to the final product **4**. Under all experimental conditions we used there is no evidence for the build-up of either a secondary radical or of the unstable epoxide, due to the absence of a peak at 1728 cm^{-1}; thus we assume that their lifetimes are very short. In the case of Fe(II) reaction, the ratio of the I(1755)/I(1717) is 1.2 while in the photolysis products is 2.8. Consequently, under the conditions in which the O−O bond is cleaved photolytically, and in the absence of bound Fe to neither of the oxygens of the endoperoxide bridge, the dominant pathway is that in which compound **5** is formed. The reaction of Fe(III) with artemisinin suggests precomplexation of Fe(III) with artemisinin, rather than a direct, reductive cleavage of the endoperoxide. This way, the first step in the reaction is displacement of a ligand from Fe(III) and Lewis acid complexation to O1 or O2. The scheme depicted in Figure 4 shows that the complexation of Fe(III) is associated with either O1 or O2. In both cases, compounds **3**, **4**, and **5** are formed. The strong line we detect at 1717 cm^{-1} as compared to the 1755 cm^{-1} indicates, in contrast to the Fe(II)-induced cleavage products and photolysis products, that the dominant species is compound **3**.

The scheme depicted in Figure 5 is based on the mechanism proposed by Posner and co-workers[4] for the activation of trioxane tosylate and is used to compare the mechanism and structures of the final products with those found in the activation of artemisinin. It shows the stages in the reaction of Fe(II) with compound **2**. The reductive cleavage of the O−O bond signals the onset of the Fe coordination to either O1 or O2. At present, the sequence of intermediates that occur at this stage of the reaction are the least well resolved, and considerable research effort is aimed at elucidating the processes that occur as the drug passes in to and out of

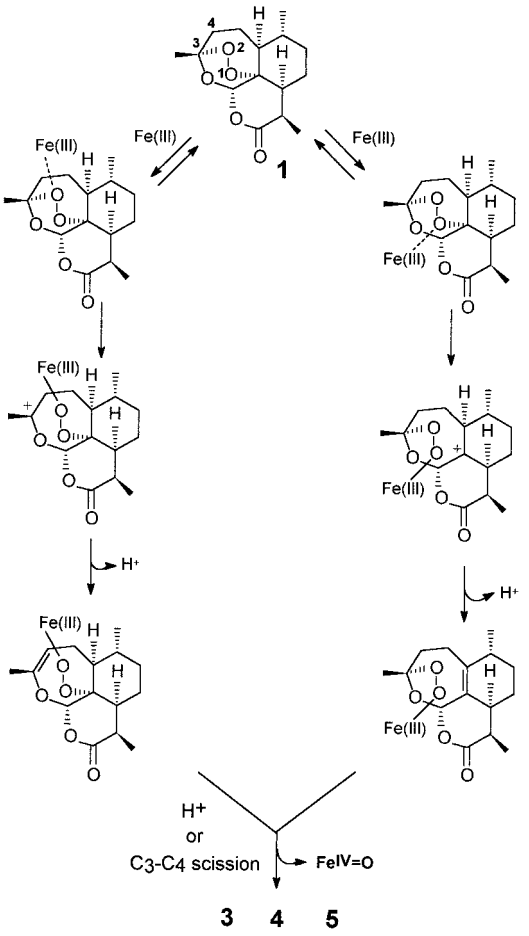

Figure 4. Proposed mechanism for the Fe(III)-induced activation of artemisinin.

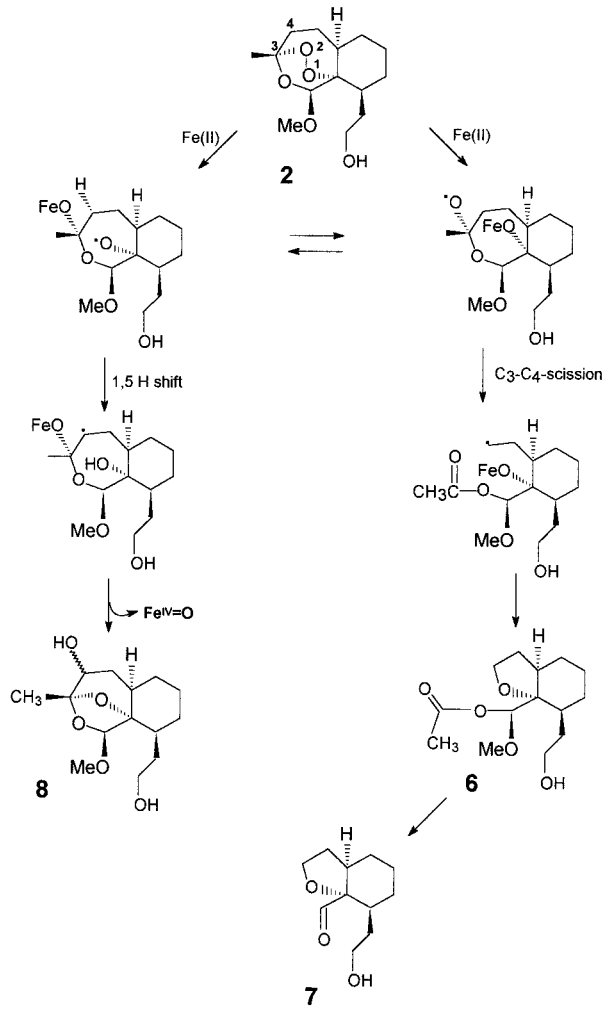

Figure 5. Proposed mechanism for the Fe(II)-induced activation of trioxane alcohol.

the Fe(III)-O level. Issues that are under active scrutiny at present include the timing of the O−O bond cleavage chemistry and the structure of the Fe(III)-OH and Fe(IV)=O intermediates. The fate of compound **6** in the reaction of compound **2** with Fe(II) contrasts sharply with that of compound **1**. The reaction in **2** proceeds to form aldehyde **7**, whereas no such product has been identified in the reaction of **1** with Fe(II). Thus, it appears that the lactone in **1** prevents conversion of the compound to an aldehyde. We suggest, in agreement with the initial proposal by Posner et al.,[4] that the 1,5-H shift process is operative in **2** with the release of Fe(IV)=O that characterizes the analogous reaction in **2**. Thus, even in the reaction of **2** with Fe(II), we suggest that there will be a branch at the initial stage and that the products distribution will be determined by the rate constant ratios, i.e., that a proportion of the reaction proceeds through the 1,5-H shift with release of Fe(IV)=O, and the remainder through the C3−C4 pathway.

In summary, the data reported here demonstrate that the reaction of Fe(II) with artemisinin is very similar to that of its synthetic analogue trioxane alcohol. In artemisin, the electron transfer from Fe(II) to initiate a cascade of radical intermediates would generate products via the 1,5-H and C3-C4 pathways. Although it was originally accepted that no Fe(III)/artemisinin reaction occurs, the measurements we have made here unequivocally demonstrate the formation of products after mixing Fe(III) with artemisinin. Finally, the observation that the endoperoxide bond is photolabile resulting in the same products as those obtained in the Fe(II) and Fe(III) reactions lays the foundation for identifying the structures of the oxygen and carbon radicals and thereby unraveling the mode of action of this fascinating class of antimalarials.

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